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THOUGHTS ON ACTION OF BOTULINUM TOXIN SUGGESTED BY REVERSIBILITY OF HEART EFFECTS

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Botulinum neurotoxin poisoning prevents the release of the neurotransmitter acetylcholine into the synaptic cleft. Stimulation of the cholinergic nerve is required for the acetylcholine-containing vesicles to attach to the inner surface at specific points of the nerve cell synaptic membrane. Thereupon at these points of attachment the acetylcholine leaves the vesicles to escape into the synaptic cleft by passage across the synaptic membrane. This event has been called exocytosis and is probably the action frustrated by the presence of toxin.

Classically botulinum neurotoxic activity has been considered to be persistent and almost irreversible. Recently cardiac effects of the toxin have been observed to be readily reversible and thus not persistent.[2,3] I suggest that this paradoxical cardiotoxicity does not challenge the theory of inhibition of cholinergic nerves and that in fact the cardiac phenomena can contribute to productive thinking about the mode of action of botulinum toxin.

Type A hemagglutinin-free toxin causes electrocardiographic changes and a decreased heart rate (bradycardia) within minutes of injection of the toxin in rodents and dogs, the species studied. Within a short time there follows a spontaneous recovery to the normal heart picture. While this spontaneous recovery is not seen *in vitro* with isolated heart preparation a rapid reversal of cardiac effects does follow simple isotonic washing out of the toxin-containing fluid bathing the isolated heart. Another significant finding is repetitious bradycardia following each of successive exposures to toxin of the isolated heart preparation after washing out of the toxin.

The persistence of bradycardia with *in vitro* isolated heart preparations is due to the continuing presence of toxin in the fluid bathing the heart. *In vivo*, after injection of toxin there is a drop in blood level as the toxin is distributed and bound to nerve cells. The heart with its apparent weaker strength of toxin binding loses the toxin to places of greater binding capacity.

Repeated *in vivo* injections of toxin result in repetition of the bradycardia-spontaneous recovery cycle. It can be adduced that spontaneous recovery by *in vivo* exposure to toxin does not mean there is any fundamental difference in nature between *in vivo* heart and *in vitro* isolated heart toxicities.

Ready reversibility of cardiac toxicity and an unimpaired capacity for toxin uptake after prior uptake and washing out of toxin suggest that the intermolecular forces binding toxin to susceptible nerve cells do not lead to profound change in the cellular physicochemical environment for nerve reception of the toxin. This is more consistent with an adsorption type

Botulinum and Tetanus Neurotoxins, Edited by B.R. DasGupta. Plenum Press, New York, 1993

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of attraction than chemical bonding, and is not indicative of an enzyme action. With enzymatic activity an exhaustion of substrate would be expected.

The exposure to toxin does not cause any discerned permanent change in the nature of the toxin and membrane receptor. Concomitantly the toxin must be bound to the synaptic membrane for the entire period of poisoning, which can be days, weeks and months long for both experimental and natural botulinum poisoning.

Two possibilities are reasonable to consider for the long-term persistence of nerve cell dysfunction. The cytochemical lesion of botulism is so profound as to resist rapid repair. The alternative I favor is that paralysis persists for as long a time as the toxin remains lodged at the synaptic membrane. The half-life of toxin in the membrane can be a long one since there are no clues to suggest rapid catabolic destruction of the embedded toxin as, for example, by proteolysis. Cytological study of repair of poisoned nerve fibrils is consistent with persistence of membrane-associated toxin. Repair is slow in becoming visible, and significantly the growth of new functional fibrils in the poisoned nerve cell takes place outside the areas of the preexisting poisoned fibrils.[1] Studies should be done for detecting continuous presence of toxin in the poisoned fibrils. This would provide definitive knowledge of the time scale of residence of toxin in the synaptic membrane and suggest if the toxin's presence hinders self-repair of the membrane site toxin occupies.

The following scenario is presented for botulinum neurotoxicity which I believe is consistent with the findings of cardiac studies.

Nerve stimulation is accompanied by two events: attachment of acetylcholine-containing vesicles to specific areas of the interior surface of the membrane and exocytosis of the vesicle. With toxin present nerve stimulation permits membrane uptake of the toxin and vesicle attachment to the synaptic membrane but exocytosis does not take place. Thus poisoned nerve cells do not accumulate acetylcholine in the cytosol. Such intracellular increase of free acetylcholine should happen if acetylcholine was released from vesicles and the released acetylcholine was not able to move across the nerve cell membrane into the synaptic cleft. Toxicity is explained if exocytosis were recognized to be a synaptic membrane–vesicle membrane interaction frustrated by toxin being present at this membrane-to-membrane interface.

Nerve stimulation acts as a valve which opens the nerve membrane to uptake of toxin. It is also accompanied by much studied intracellular and synaptic membrane changes in ionic environment in which calcium is a major participant. Conceivably toxin in the synaptic membrane disturbs access of ions to the trigger spot for exocytosis at the synaptic membrane–vesicle membrane interface. Exocytosis could be prevented by the toxin physically masking the trigger spot. This prevents contact of the proper ions with the trigger spot. Removal of the toxin which yields reversible cardiac toxicity means that the toxin resident in the synaptic membrane causes no irreversible changes in the events associated with initiation of exocytosis and movement of acetylcholine across the synaptic membrane. This is consistent with rapid recovery following removal of the toxin.

An explanation of botulism poisoning must account for differences in sensitivity to toxin among the sympathetic, parasympathetic and central nervous systems in spite of some common possession of cholinergic neurons. There might be differences among these systems in barriers affecting diffusion of toxin from lymph into the synaptic cleft. There may be a limiting width of the synaptic cleft that must be exceeded to allow entrance of the large-sized 150,000 dalton molecular weight toxin, a factor which might operate in the densely packaged brain. The cardiac effects at this stage of knowledge tell us nothing about the possibilities.

A prime factor affecting toxicity would be varying strength of binding forces of synaptic membranes holding toxin. These binding forces could differ among cholinergic neurons associated with different parts of body tissues and organs. If there is a spectrum from high- to low-strength binding forces the heart would appear to represent a marginal- or low-strength binding entity. A task that needs to be undertaken is to identify the sites and nature of toxin-

susceptible cardiac innervation. What can be said is that the toxin does not affect the intrinsic automaticity of the beat of isolated heart cells. It can be said that toxin inhibition of heart rates rests on nervous system control at sites located in the heart.

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